# INTERMEDIATES OF STIGMASTEROL METABOLISM IN *SPODOPTERA LITTORALIS*

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## ABSTRACT

Stigmasterol-24,28-epoxide, 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol, and 22E-cholesta-5,22,24-trien-3$\beta$-ol were identified as normal metabolites of [$^3$H]stigmasterol in *Spodoptera littoralis* larvae. Relative concentrations of all three of these metabolites increased when a diazasterol inhibitor was fed in combination with stigmasterol in the artificial diet. Identification of these sterols as intermediates in the conversion of stigmasterol to cholesterol in this insect indicates that intermediates analogous to fucosterol and fucosterol-24,28-epoxide in the conversion of sitosterol to cholesterol are produced in the metabolism of stigmasterol. This is the first published identification of stigmasterol-24,28-epoxide and 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol as intermediates in this pathway in an insect.

## INTRODUCTION

Insects are unable to biosynthesize sterols and require a dietary source to support development and reproduction (1). The ability of many phytophagous and omnivorous insect species to convert 28- and 29-carbon phytosterols to cholesterol by dealkylation of the side chain is essential to provide the cholesterol required for molting hormone (ecdysteroid) synthesis (2). Thus, a unique area of insect biochemistry is available for exploitation in the development of new, safe, selective control technology. It has been shown that certain inhibitors can disrupt the conversion of phytosterols to cholesterol and larval development in a number of plant feeding species including the

tobacco hornworm, *Manduca sexta*, the fall armyworm, *Spodoptera frugiperda* (3), the silkworm, *Bombyx mori* (4), and the cotton leafworm, *Spodoptera littoralis* (5). With considerable in-depth knowledge of the pathways involved in the biochemical conversion of phytosterols to cholesterol in insects it may be possible to discover more potent inhibitors that would be sufficiently selective and environmentally compatible.

Sitosterol and stigmasterol are two of the most ubiquitous phytosterols, and of the two, sitosterol metabolism in insects has been examined in greatest detail. The metabolic sequence sitosterol → fucosterol → desmosterol → cholesterol was elucidated in studies with *M. sexta* (6) and desmosterol was shown to be an intermediate in this pathway in a number of insect species (3). It was also established that desmosterol was a common intermediate in the conversion of a variety of $C_{28}$ and $C_{29}$ phytosterols to cholesterol in insects (6). The metabolism of phytosterols (particularly sitosterol and campesterol) in the silkworm was shown to be similar to that in *M. sexta* (4). In addition, fucosterol 24,28-epoxide was determined to be an intermediate following fucosterol in this pathway in *B. mori* (4) and several other species, as reviewed in detail by Rees (5).

The dealkylation and conversion of stigmasterol to cholesterol was examined in some depth in *M. sexta* where 22E-cholesta-5,22,24-trien-3$\beta$-ol was determined to be an intermediate in the pathway prior to desmosterol. Both sterols accumulated in the insect tissues when the inhibitor 20,25-diazacholesterol was included in the artificial diet (7). Unpublished

metabolic studies with the cotton leafworm, *Spodoptera littoralis*, indicated that the dealkylation of stigmasterol occurs similarly to the pathway in *M. sexta* (involving $\Delta^{5,22,24}$-triene and desmosterol intermediates) and this pathway appears to be analogous to the conversion of sitosterol to cholesterol, i.e., involving epoxide and 24-ethylidene intermediates (5). In order to establish unambiguously the involvement of stigmasterol-24,28-epoxide and 22E-cholesta-5,22,24-trien-3$\beta$-ol as intermediates and to verify the involvement of the suspected intermediate 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol in this pathway in *S. littoralis*, we examined the metabolism of stigmasterol ($^{3}$H-labeled and unlabeled) included in an artificial diet both with and without an azasteroid inhibitor.

## MATERIALS AND METHODS

Biological material: Insects from a laboratory strain of *S. littoralis* routinely reared on an artificial agar-based diet, as previously described (8), was used. Stigmasterol was purchased from Koch-Light Laboratories Ltd., Haverhill, Suffolk, U.K. [2,4-$^{3}$H]Stigmasterol, prepared as previously described (9), was diluted to a specific activity of 220 dpm/$\mu$g and determined to have 96.7% radiochemical purity by thin-layer chromatography (TLC). 20,25-Diaza-cholesterol · dihydrochloride was a gift of the G. D. Searle Co., Chicago, IL. 22E-Cholesta-5,22,24-trien-3$\beta$-ol was prepared as previously described (10) and 22E-stigmasta-5,22,24(28Z)-trien-3$\beta$-ol was synthesized via published methods (11). The acetate of the E isomer was prepared via reaction of cholesta-5,22-dien-24-keto-3$\beta$-yl acetate with ethyl magnesium bromide and subsequent dehydration of the 24-ol compound with acetic anhydride at reflux temperature. For our experiments, either radiolabeled or unlabeled stigmasterol was coated on the dry components of the diet by adding the sterols in dichloromethane solution, and stirring until the solvent was evaporated. The azasteroid, 20,25-diazacholesterol · dihydrochloride, was added in a similar manner but required adding sufficient methanol to dissolve the azasteroid before adding dichloromethane. Radiolabeled or unlabeled stigmasterol as well as the azasteroid were each present in the diet at a final concentration of 0.026% (wet weight). To obtain uninhibited insects, in addition to diets containing dietary sterol in combination with the azasteroid inhibitor, insects

were fed diets without the azasteroid.

Newly-hatched larvae were fed diets containing labeled or unlabeled stigmasterol until they were mature sixth-instar larvae. They were then fed only an agar-water gel for 24 hours to clear the digestive tract of dietary sterols before being weighed and stored in methanol in a freezer until extraction. In order to obtain sufficient material from insects fed diets containing the azasteroid inhibitor, the larvae were fed diet without inhibitor until the third-instar. At that time, they were placed on the azasteroid-containing diet until they were mature sixth-instar larvae and then they were transferred to the agar gel, harvested and stored as described above.

Extraction and sterol isolation: The methanol in which the insects were stored was decanted off, dried on the rotoevaporator, and the residue sequestered to be combined later with the extracted material. The insects were homogenized and extracted in acetone, filtered and extracted twice more with acetone. The acetone extracts were combined, reduced in volume, the residue from the methanol decanted earlier from the insects was introduced, and diethyl ether was added. This was partitioned against water, and the aqueous phase was partitioned three more times against ether. The ether extracts were combined and washed three times with water. The ether phase was dried over sodium sulfate, filtered and evaporated to dryness on the rotoevaporator. The crude lipid was saponified under reflux in ethanolic 6% (w/v) potassium hydroxide with 1% pyrogallol antioxidant added. A sample of stigmasterol control diet was also extracted and the sterols isolated and purified in the same manner as the insect sterols.

The nonsaponifiables were extracted with ether (3x) after dilution of the saponification mixture with water. The ether phases were combined and washed to neutrality with water and then dried over sodium sulfate, filtered and taken to dryness on the rotoevaporator. The nonsaponifiables were chromatographed on neutral grade III Woelm alumina eluted with the following solvent fractions: petroleum ether; 2, 6, 9, and 35% diethyl ether in petroleum ether, respectively; and diethyl ether. The bulk of the sterols eluted in the 35% fraction, but a small portion was usually found in the ether fraction as well, as determined by monitoring fractions by thin-layer chromatography (TLC).

Sterol epoxide was separated from the other insect sterols by preparative TLC on silicic acid plates (Kieselgel G60, 0.5 mm) developed in 30% ethyl acetate in hexane. The sterol epoxide and "other" sterol bands were visualized by spraying the plates with berberine sulfate and viewing under UV light. Authentic fucosterol-24,28-epoxide ($R_f$=0.34) and cholesterol ($R_f$=0.41) were used as standards to identify the bands. The sterol epoxide and "other" sterol bands were scraped from the plates and eluted with diethyl ether.

The recovered sterols (other than epoxides) were acetylated in pyridine/acetic anhydride (2:1 v/v) and fractionated by argentation chromatography on 20% $AgNO_3$-impregnated silicic acid (Kieselgel 60, Merck). Fractions were eluted with: petroleum ether; 5, 10, 15, and 20%

diethyl ether in petroleum ether, respectively; and diethyl ether. Steryl acetate fractions were monitored on 10% $AgNO_3$-impregnated Kieselgel G60 chromatoplates developed in hexane/chloroform (30:70).

Sterol identification: Free sterols and steryl acetates were analyzed by capillary gas-liquid chromatography (GLC) in a Hewlett-Packard model 5890A gas chromatograph equipped with a flame-ionization detector, and interfaced with a H-P model 3393A integrator. Identification was by comparison of retention times relative to cholestane (RRT's) of insect sterols and their acetates with authentic sterols and their acetates. The capillary column was a J & W DB-1 fused silica column, 15 m x 0.241 mm i.d. (0.25 $\mu$m film), at 280 C oven temperature, using helium carrier gas, and 20:1 split ratio.

High-performance liquid chromatography (HPLC) was performed with a Waters model 501 system on a Beckman Ultrasphere ODS column (4.6 mm x 25 cm; 5 $\mu$ particle size). Absorbance was monitored at 214 nm with a Waters model 441 absorbance detector and automatically recorded with a Trivector Trio Data Processor. Isocratic elution with methanol (1.0 ml/min flow rate) was used for steryl acetates injected in methanol/ethyl acetate (3:1) and a methanol/water/tetrahydrofuran (88:7:5) system was used for sterol epoxides. Fractions of unknown steryl acetates or sterol epoxides of sufficient purity were collected via HPLC for mass spectral and nuclear magnetic resonance (NMR) analyses of 22E-cholesta-5,22,24-trien-3$\beta$-yl acetate, 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate and stigmasterol-24,28-epoxide. Certain fractions were further fractionated and analyzed to identify 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate on a Spectra Physics model SP8700 system equipped with a Waters 990 Photodiode Array Detector (monitored at 202 and 235 nm).

Steryl acetates were analyzed by gas chromatography-mass spectrometry (GC-MS) with a VG 7070F mass spectrometer linked to a Finnigan Incos 2300 Data System equipped with a Pye Unicam model 204 gas chromatograph. The GC column was an SGE BP-5 fused silica capillary column, 12 m x 0.25 mm i.d. (0.25 $\mu$m film) programmed ambient to 150 C ballistically, then 12°/min to 280 C. Stigmasterol-24,28-epoxide was analyzed on the same mass spectrometer, but samples were introduced via probe (220 C source).

Fourier transform NMR spectra were recorded on a Bruker 400.13 MHz instrument (SERC High Field NMR Service, Dept. of Chemistry, University of Warwick) or a General Electric QE-300 NMR spectrometer (Beltsville, MD). Samples were analyzed in $CDCl_3$ except the data for 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate was obtained in $C_6D_6$. Radioactive samples were counted on an LKB model 1219 Rackbeta liquid scintillation counter. UV spectra were obtained on a Perkin-Elmer Model 559 UV-VIS Spectrophotometer.

## RESULTS

The most abundant sterols occurring in the sterol samples isolated from *S. littoralis* (cholesterol, desmosterol, campesterol, stigmasterol, and sitosterol) were easily identified by comparison of GLC RRTs both as the free sterols and as their acetates, HPLC retention times and by GC-MS of the acetates. The relative percentages of sterols from insects fed stigmasterol-fortified diets both with and without the azasteroid inhibitor are summarized in Table 1.

TABLE 1. RELATIVE PERCENTAGES OF *SPODOPTERA LITTORALIS* STEROLS EXCLUDING EPOXIDE BAND*

| | Dietary Supplements | |
|---|---|---|
| Sterol | [$^3$H]Stigmasterol | [$^3$H]Stigmasterol + 20,25-Diazacholesterol |
| Cholesterol | 59.2 | 11.6 |
| Desmosterol | 15.0 | 8.0 |
| 22E-Cholesta-5,22,24-trien-3$\beta$-ol | Tr‡ | 6.1 |
| Campesterol | 6.3 | 22.4 |
| Stigmasterol | 10.7 | 21.9 |
| 22E-Stigmasta-5,22,24(28E)-trien-3$\beta$-ol | Tr | 4.2 |
| Sitosterol + Fucosterol | 8.5 | 25.8 |

* Relative percentages of major sterols from stigmasterol control diet were as follows: Stigmasterol 72.5, Sitosterol 15.6, Campesterol 4.8 and Cholesterol 1.6%, respectively.

‡ Tr < 0.4%

Stigmasterol-24,28-epoxide was isolated by chromatographing the sterols in the 35% diethyl ether in petroleum ether fraction from alumina column chromatography. The epoxide band separated by TLC from all other

sterols was found to include some 22E-cholesta-5,22,24-trien-3$\beta$-ol in addition to stigmasterol-24,28-epoxide, particularly in samples from insects fed the azasteroid inhibitor, where it was the major component. When aliquots of fractions from [$^3$H]stigmasterol-fed insects were collected from HPLC of the epoxide band sterols and counted, radioactivity was found to be associated with both the stigmasterol-24,28-epoxide and the 22E-cholesta-5,22,24-trien-3$\beta$-ol peaks, with no significant labeling associated with any other components. Much less 22E-cholesta-5,22,24-trien-3$\beta$-ol relative to stigmasterol-24,28-epoxide was present in the epoxide band from sterols isolated from uninhibited insects. Most of the $\Delta^{5,22,24}$-trien compound was isolated from TLC with all the other, less polar sterols, but the $R_f$ of the sterol was such that some of it was recovered in both bands from all samples isolated. Sufficiently pure samples of stigmasterol-24,28-epoxide and 22E-cholesta-5,22,24-trien-3$\beta$-ol were obtained from HPLC fractions for NMR and MS analyses.

The MS for stigmasterol-24,28-epoxide from *S. littoralis* (direct probe introduction) included (expanded 10x) an $M^+$ of m/z 426 (3%) and fragments at 408(M-$H_2O$, 80), 271(M-SC-2H, 18), and 255(M-SC-18, 100). Data from the $^1$H-NMR spectrum for stigmasterol-24,28-epoxide are listed in Table 2 for comparison with data from 22E-cholesta-5,22,24-trien-3$\beta$-ol isolated from *S. littoralis*.

The sterol suspected to be 22E-cholesta-5,22,24-trien-3$\beta$-ol trapped from HPLC had a GLC RRT identical to the authentic sterol. The MS for

22E-cholesta-5,22,24-trien-3$\beta$-ol from *S. littoralis* included (expanded 7x) an $M^+$ of m/z 382(18%), 364(M-$H_2O$, 28), 300(M-82, 33), 271(M-SC-2H, 98), 253(M-SC-2H-$H_2O$, 100), and a strong 109 peak without enhancement indicated side chain cleavage. These results compared very well with the MS of the authentic sterol and with MS data from Hutchins *et al.* (10). $^1$H-NMR data for this sterol from the insect is summarized in Table 2.

TABLE 2. $^1$H-NMR SIGNALS FOR *SPODOPTERA LITTORALIS* STEROLS

| | Sterol | | |
|---|---|---|---|
| $^1$H | Stigmasterol 24,28-epoxide | 22E-Cholesta-5,22,24-trien-3$\beta$-ol | 22E-Stigmasta-5,22,24(28E)-trien-3$\beta$-ol* |
| | $\delta$ | $\delta$ | $\delta$ |
| 18Me | 0.692(s) | 0.695(s) | 0.665(s) |
| 19Me | 0.998(s) | 0.998(s) | 0.928(s) |
| 21Me | 1.044(d) J=6.8Hz | 1.032(d) J=6.4Hz | 1.150(d) J=6.6Hz |
| 26/27 Me's | 0.912(d) J=6.8Hz<br>0.960(d) J=7.2Hz | 1.727(d) J=6.4Hz | 1.161(d) J=6.9Hz |
| 29Me | 1.161(d) J=5.6Hz | | 1.736(d) J=7.2 Hz |
| C-6-H | 5.338(m) | 5.343(m) | 5.352(m) |
| C-22-H | 5.473(d) J=8.4Hz<br>5.512(d) J=8.4Hz | 5.374(d)broad<br>5.413(d)broad | 5.580(d) broad<br>5.632(d) broad |
| C-23-H | 5.397(d) J=15.6Hz | 6.105(d)broad<br>6.143(d)broad | 6.375(d) J=15.6 |
| C-24-H | | 5.741(d)broad | |
| C-3-H | 3.515(m) | 3.640(m) | 4.872(m) |
| C-25-H | 2.931(q) J=5.2Hz | | 2.674(sept) |
| C-28-H | | | 5.398(quartet) |
| $CH_3CO$ | | | 1.757(s) |

*NMR data obtained on the acetate in $C_6D_6$.

22E-Cholesta-5,22,24-trien-3$\beta$-yl acetate from insect sterols (particularly from inhibited insects) was most abundant in fraction 6 (diethyl ether) from silicic acid argentation chromatography. The MS from GC-MS of this steryl acetate included an $M^+$ of m/z 424(5%), 364(M-$CH_3COOH$, 42), 349(M-$CH_3COOH$-$CH_3$, 24), 253(M-$CH_3COOH$-SC-2H, 38), and 109(SC, 100). These ions matched data from MS of authentic standard and data from Hutchins *et al.* (10). In addition, the RRT from GLC of the acetate of the $\Delta^{5,22,24}$-compound from the insect matched the RRT for the authentic standard. All analytical data indicate that the insect sterol is identical to authentic 22E-cholesta-5,22,24-trien-3$\beta$-ol.

For analytical purposes, the best yield of suspected 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate from insect sterols was obtained from fraction four (and sometimes fraction 5) from argentation-silicic acid chromatography of acetates from sterols isolated from azasteroid-inhibited insects. Such samples were further fractionated by rechromatography on the same type of column and this produced material for HPLC from which quite pure steryl acetate could be collected. In HPLC fractions from insects fed [$^3$H]stigmasterol without azasteroid, a major portion of radioactivity was found to be associated with 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate.

The MS for the acetate of 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol from insect sterols included an $M^+$ of m/z 452($M^+$, very weak), 392(M-$CH_3COOH$, 18%), 313(M-SC-2H, 13), and 137(SC, 55). The capillary GLC RRTs of the $\Delta^{5,22,24(28)}$-compound and its acetate from insects and the synthetic sterol

and its acetate are compared with stigmasterol, sitosterol, and isofucosterol and their acetates in Table 3. GLC of a mixture of the acetate of the sterol isolated from the insect and authentic 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-yl acetate yielded a single peak. There was only a slight difference in GLC RRTs of the E and Z isomers, but they separated satisfactorily as the acetates ($R_f$ of Z = 0.41; $R_f$ of E = 0.47) upon argentation chromatography when the chromatoplate was developed in hexane-toluene (3:2) three times. The $R_f$ of the acetate of the insect sterol corresponded to that of the authentic E isomer. Typical absorption ($\lambda_{max}$=236nm) expected of this conjugated diene system in the side chain, comparable to synthetic standard, was seen upon UV analysis. $^1$H-NMR spectral data for the acetate of this sterol from the insect is summarized in Table 2 and is identical to data from authentic 22E-stigmasta-5,22,24(28E)-3$\beta$-yl acetate.

TABLE 3. RELATIVE RETENTION TIMES OF SYNTHETIC 22E-STIGMASTA-5,22,24(28E)-TRIEN-3$\beta$-OL, STEROL ISOLATED FROM *SPODOPTERA LITTORALIS*, AND THEIR ACETATES COMPARED TO RRT'S OF STIGMASTEROL, SITOSTEROL AND ISOFUCOSTEROL AND THEIR ACETATES

| | RRT | |
|---|---|---|
| Sterols | Free | Acetate |
| Stigmasterol | 2.60 | 3.53 |
| 22E-Stigmasta-5,22,24(28E)-trien-3$\beta$-ol (insect) | 2.81 | 3.74 |
| 22E-Stigmasta-5,22,24(28E)-trien-3$\beta$-ol (synthetic) | 2.79 | 3.76 |
| Sitosterol | 3.01 | 4.01 |
| Isofucosterol | 3.12 | 4.12 |

## DISCUSSION

Data from these studies provide considerable information on the intermediates involved in the conversion of stigmasterol to cholesterol in *S. littoralis*. Previous studies with this insect have shown that desmosterol, fucosterol, and fucosterol-24,28-epoxide are intermediates in the conversion of sitosterol to cholesterol (5,12). Additional evidence indicated that a 24,28-epoxide and a 22E-cholesta-5,22,24-trien-3$\beta$-ol may be involved as intermediates in the dealkylation and conversion of stigmasterol to cholesterol (5). In-depth metabolic studies with *Bombyx mori* have shown fucosterol and its epoxide to be intermediates in the conversion of sitosterol to cholesterol and also that similar side chain alterations occur in the conversion of campesterol to cholesterol in *B. mori* (4). Stigmasterol metabolism studies with the Mexican bean beetle, *Epilachna varivestis*, provided evidence that a $\Delta^{22,24}$-diene and a $\Delta^{24}$-side chain compound occur as intermediates even though the $\Delta^{5}$-bond is saturated by the insect before dealkylation (13).

It is of interest to note the relatively high level of desmosterol (15%) normally found in uninhibited *S. littoralis* sterols in these studies with stigmasterol added to the diet (Table 1). In other phytophagous insect species that are capable of dealkylation, e.g. *M. sexta*, desmosterol comprises 1-2% of the total sterols when insects are fed diets with sitosterol, stigmasterol or other phytosterols included as dietary sterols (14). Less desmosterol (8%) accumulated in the sterols of *S. littoralis* fed the diazacholesterol in combination with stigmasterol than in uninhibited insects. There also is

considerably less cholesterol in control *S. littoralis* sterols than is found in other insects fed stigmasterol as the dietary sterol. Even with the addition of the diazasterol inhibitor to the diet in the 3rd instar, the total dealkylated sterol is decreased by *ca.* two-thirds.

As found in earlier studies with other species (6), addition of an azasteroid inhibitor to the insect diet caused accumulation of certain intermediates in the insect tissues. All three of the stigmasterol metabolites of interest (stigmasterol-24,28-epoxide, 22E-cholesta-5,22,24-trien-3$\beta$-ol and 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol) were present in considerably higher concentrations in sterols from insects fed diets containing the diazasterol than in sterols from uninhibited insects, thus facilitating their isolation in suitable quantities for identification. However, all three of these intermediates were identified also in sterols from insects fed stigmasterol without diazasterol and all were radiolabeled when [$^3$H]stigmasterol was fed.

Argentation chromatography by TLC provides strong evidence that the $\Delta^{5,22,24(28)}$-sterol from *S. littoralis* has the same configuration of the ethylidene moiety as fucosterol (28E). Further, the RRT's of the synthetic $\Delta^{5,22,24(28E)}$-sterol and its acetate compared most favorably with RRT's of the insect sterol and its acetate. Thus, the GLC data (Table 3) also indicate that the configuration of the 24-ethylidene of 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol is the same as fucosterol. Isofucosterol separates well from sitosterol on capillary GC, eluting after sitosterol, whereas fucosterol coelutes with sitosterol. However, as either the free or acetylated sterols the Z and E

isomers of the $\Delta^{5,22,24(28)}$-sterols nearly coelute. In addition, a septet centered at 2.88 ppm in the NMR spectrum of the acetate of the synthetic Z isomer is indicative of a C-25 isopropyl methine proton of a 24-ethylidene sterol possessing the configuration of isofucosteryl acetate. On the other hand, the isopropyl methine proton reportedly gives a septet at 2.2 ppm for those ethylidene sterols with the configuration like fucosterol (E isomer) or its acetate (15). However, with our synthetic E isomer and the insect sterol this proton resonance was centered at 2.674 ppm (Table 2).

Another important aspect of the present study was the isolation and identification of stigmasterol-24,28-epoxide. Although it is easily separated by TLC from most of the other sterols, the epoxide does not usually survive GLC analysis and most fractionations of sterols from insects have included acetylation (before argentation chromatography), which also destroys this sterol. Thus, this indicates that the examination of insect sterols by HPLC should be an early step in their analysis.

The present report provides strong evidence that the following metabolic pathway exists in *S. littoralis*: stigmasterol → 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol → stigmasterol-24,28-epoxide → 22E-cholesta-5,22,24-trien-3$\beta$-ol → desmosterol → cholesterol. This is the first published data on the identification of 22E-stigmasta-5,22,24(28E)-trien-3$\beta$-ol and stigmasterol-24,28-epoxide as metabolites of stigmasterol and intermediates in the conversion of stigmasterol to cholesterol in an insect, although there has been indirect evidence presented for *B. mori* (16). Thus, intermediates

analogous to fucosterol and fucosterol-24,28-epoxide which occur in the conversion of sitosterol to cholesterol are also involved in stigmasterol metabolism in *S. littoralis*.

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NOMENCLATURE

cholesterol: cholest-5-en-3$\beta$-ol; desmosterol: cholesta-5,24-dien-3$\beta$-ol; sitosterol: 24$\alpha$-ethylcholest-5-en-3$\beta$-ol; campesterol: 24$\alpha$-methylcholest-5-en-3$\beta$-ol; stigmasterol: 24$\alpha$-ethylcholesta-5,22E-dien-3$\beta$-ol; fucosterol-24,28-epoxide: 24,28-epoxystigmast-5-en-3$\beta$-ol; stigmasterol-24,28-epoxide: 24,28-epoxystigmasta-5,22-dien-3$\beta$-ol; fucosterol: 24E-ethylidenecholest-5-en-3$\beta$-ol; isofucosterol: 24Z-ethylidenecholest-5-en-3$\beta$-ol.

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